Cardiovascular, pharmacology and chemistry
Committee:
Dr. Jacobson, Chairman
Dr. Cattell
Dr. Bing

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TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Application for Research Grant

Date: July 20, 1960

1. Name of Investigator: Paul S. Larson, Ph.D.

2. Title: Professor of Pharmacology

3. Institution & Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject: The effect of tobacco smoke and nicotine on ascorbic acid metabolism.

5. Detailed Plan of Procedure:

Numerous reports have included claims for an effect of tobacco smoke and specifically nicotine on ascorbic acid metabolism. Representative of these, and some to the contrary, are the following.

Decreased urinary excretion of ascorbic acid in smokers (Strauss & Scheer, Zschr. Vitamin-forsch. 9:39, 1939; Goyanna, Brasil med. 69:173, 1955). Increased followed by decreased excretion in the urine in mice exposed to cigarette smoke for 20 minutes a day (Venulet & Moskwa, Polski Tygod, Lekarski 7:281, 1952).

Decreased ascorbic acid content in the milk of smoking mothers (Venulet & Danysz, Acta Physiol. Polonica 4:646, 1954; Pediat. Polska 30:811, 1955).

Lowered blood ascorbic acid levels in smokers or from smoking tests (Harmsen, Deut, med. Wschr. 67, 790, 1941; Venulet & Moskwa, Polski Tygod. Lekarski, 7:281, 1952; Bourquin & Musmanno, Am. J. Digest. Dis. 20:75, 1953). However, Hogler (Wien. med. Wschr. 93:309, 1943) in studies on more than 100 smokers and non-smokers found no regular differences in fasting ascorbic acid blood-levels, even in acute tests (smoking 3 cigarettes in succession on an empty stomach). Also, Wenzel and Beckloff (J. Am. Pharmaceut. Assoc., Sci. Ed. 47:338, 1958) did not find significant differences in the serum ascorbic acid levels of rabbits maintained on control diet or fed 0.1% cholesterol in the diet, or given 2.8 mg./kg./day nicotine in the drinking water, or given combined cholesterol and nicotine.

Decreased adrenal ascorbic acid content following nicotine administration (rat: Maren, Proc. Soc. Exp. Biol. Med. 77:521, 1951). Decreased adrenal ascorbic acid content following exposure to tobacco smoke (frog: Venulet & Moskwa, Polski Tygod, Lekarski 7:281, 1952; mouse: Venulet & Majcherski, ibid 10:6, 1955; guinea pig and rabbit: Lupu, Velican & Mihaescu, Session of Med. Sci. Section of Roumanian Acad. of 22-24 Jan. 1954, p. 141-168).

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In in vitro studies, Reif (Biochem. Z. 315:310, 1943) found that destruction of ascorbic acid by nicotine proceeded only slowly in pure distilled water but could be markedly accelerated by small amounts of copper. With small amounts of nicotine the reaction stopped at the dehydroascorbic acid stage; with large amounts, no ascorbic acid was recoverable by treatment with hydrogen sulfide. Bourquin & Musmanno (1953 reference above) added to blood of known ascorbic acid content, nicotine in amount estimated to equal the amount which might be absorbed in heavy smoking and in 3 experiments found that 24.4 - 31.6% of the original ascorbic acid had been lost after 5 or 10 minutes.

If this effect indeed occurs, it would seem well to learn more concerning its magnitude and its mechanism, so as to help judge its possible physiological importance to the body economy, since the vitamin has been implicated in steroid and cholesterol synthesis, iron metabolism, the degradation of tyrosine, collagen formation, cellular respirations, and as a factor in the bodies resistance to stress.

Proposed Research

Analysis of 24-hour urine samples of large groups of smoking and nonsmoking students and volunteers using the colorimetric method of Roe and Kuether (J. Biol. Chem. 147:399, 1943) which measures both forms of the vitamin by the color produced by reaction with 2,4-dinitrophenyl-hydrazine after oxidizing the reduced form by treatment with "Norit" and acetic acid. The assay commonly used by the workers cited above was the titrimetric decolorization of the dye, 2,6-dichlorophenol indophenol by the reducing action of l-ascorbic acid. This does not account for the oxidized form (which also has antiscorbutic activity) unless first reduction with hydrogen sulfide is effected, a precaution mentioned by only one of the investigators cited (Reif).

Observations on ascorbic acid excretion in "ascorbic acid loaded" volunteers during period of abstinence, smoking regular and possibly denicotinized cigarettes, and in nonsmokers.

Animal studies using both species that can synthesize ascorbic acid and ones that cannot. Particular emphasis will be placed on the use of C^{14} labeled ascorbic acid to follow the excretion of C^{14} as well as ascorbic acid, with and without exposure to nicotine, as well as preparing autoradiograms of urine following chromatographic development in suitable solvent systems.

In vitro studies on ascorbic acid and nicotine combinations in aqueous and blood serum media using both cold and C^{14} ascorbic acid. Rate of disappearance of ascorbic acid will be followed and autoradiograms will help determine the formation of chemical transformations.

Further direction will depend on findings during the course of the above studies.

6. Budget Plan: 1st year

Salaries	2,550.
Expendable Supplies	2,000.
Permanent Equipment	--
Overhead (10%)	470.
Other (Travel)	150.
Total	$5,170.

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7. Anticipated Duration of Work: Two years (The second year budget should be about the same as the first year one.

8. Facilities and Staff Available:

Facilities include spectrophotomer, chromatographic and C^{14} counting equipment needed for the study. Mr. Robert I. Toborowsky will spend virtually full time on the project. Mr. Toborowsky is a graduate student in his second year of study for the Ph.D. degree. He is an outstanding student with a straight "A" record and the study will be his thesis research. $2,350 of the salary item is to be for his fellowship stipend ($2,000) and tuition costs ($350); the remaining $200 is to contribute toward animal technicians salaries. Dr. Larson and the other members of our staff will contribute in supervisory capacities.

9. Additional Requirements: None

10. Additional Information:

The possibility exists that relationships to nicotine metabolism studies in progress will develop from this study.

Signature Paul S. Larson
Director of Project

R. Blackwell Smith Jr.
President

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